THE EFFECT OF CERTAIN ORGANIC AND INORGANIC SUBSTANCES UPON LIGHT PRODUCTION BY LUMINOUS BACTERIA.

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While engaged in a study of the chemistry of light production by luminous bacteria I had occasion to investigate the effect of diluting the sea water with distilled water and with isotonic sugar solution and the influence of the various salts of sea water, of acids and alkalies, and of certain anæsthetics upon the emission of light. The results are of interest for comparison with the known effects of these substances on other organisms and with other vital manifestations of life.

In all experiments, except where otherwise noted, one drop of the dense emulsion of luminous bacteria (a form isolated from squid at Woods Hole, Mass.) was added to 30 c.c. of solution in an uncorked Erlenmeyer flask and the whole thoroughly mixed. For comparative observations it is essential that the eye be thoroughly adapted to the dark and that each flask be oxygenated by shaking, before judging as to the emission or absence of light. Observations were made after 10 minutes, one hour and 24 hours.

TABLE I.

EFFECT OF DILUTION OF SEA WATER WITH WATER AND WITH *m* CANE SUGAR SOLUTION.

Dilution with Water.					Dilution with *m* Cane Sugar.				
Parts Sea Water.	Parts Water.	Light after			Parts Sea Water.	Parts *m* Sugar.	Light after		
		10 Min.	1 Hr.	24 Hrs.			10 Min.	1 Hr.	24 Hrs.
2	1	+	+	+	2	1	+	+	+
1	1	faint	faint	−	1	1	+	+	+
1	2	faint	very faint	−	1	2	+	+	+
1	4	faint	−	−	1	4	+	+	faint
1	6	very faint	−	−	1	6	+	+	faint
1	10	−	−	−	1	10	+	+	faint
1	14	−	−	−	1	14	+	+	−
1	20	−	−	−	1	20	+	+	−
Sea water		+	+	+	*m* cane sugar		+	+	−
Water		−	−	−					

It will be noted from the above Table I. that the bacteria cease to give off light and experiment shows that they are killed by too great dilution with water. That this effect is not entirely due to the absence of salt but is chiefly due to a cytolysis through lowered osmotic pressure is shown by diluting the sea water with an inert isotonic solution, cane sugar. Some salt is necessary for the continued production of light as the bacteria no longer glow after twenty four hours' emersion in *m*-sugar, a fact of no great surprise as unicellular freshwater luminous animals are unknown.

TABLE II.

EFFECT OF ACID AND ALKALI.

Conc. of Acid and Alkali Added to Mg-free Sea Water, *m*/2 (100 NaCl+2.2 KCl+2$CaCl_2$) in Syracuse Watch Glasses.	Light after		
	10 Min.	1 Hr.	24 Hrs.
n/2000 HCl	−	−	−
n/4000 HCl	faint	−	−
n/8000 HCl	+	faint	−
n/16000 HCl	+	+	−
n/32000 HCl	+	+	faint
n/500 valerianic acid	−	−	−
n/1000 " "	faint	−	−
n/2000 " "	faint	−	−
n/4000 " "	+	faint	−
n/8000 " "	+	+	−
n/16000 " "	+	+	faint
n/500 NaOH	−	−	−
n/1000 NaOH	−	−	faint[1]
n/2000 NaOH	+	+	+
n/250 methyl amine	−	−	−
n/500 " "	faint	−	faint[1]
n/1000 " "	faint	+[1]	faint
n/2000 " "	+	+	+
Mg-free sea water	+	+	faint
Sea water	+	+	+

[1] Probably due to neutralization of alkali through absorption of CO_2.

As was to be expected acids and alkalies prevent light emission in very weak concentration, the acids in much weaker concentration than the alkalies. In fact the bacteria are very sensitive to acid and will not even phosphoresce with any brilliancy in a neutral medium.

The organic acid (valerianic) and alkali (methyl amine) have less effect than the inorganic, a result at variance with my results for other organisms which are usually affected more readily by the weak than by the strong acids and alkalies.[1]

[1] Harvey, E. N., "Studies on Acids," in Carnegie Institution Publications No. 212, p. 143, 1915; on alkalies, *id.*, No. 183, p. 131, 1914.

TABLE III.

EFFECT OF VARIOUS COMBINATIONS OF THE SALTS OF SEA WATER.

Salt Combinations.	Light after		
	10 Min.	1 Hr.	24 Hrs.
Sea water	+	+	+
Artificial Sea Water, $m/2$ (100 NaCl+2.2 KCl+ 2 $CaCl_2$+10 $MgCl_2$)+$n/4000$ NaOH	+	+	+
Neutral artificial sea water	+	+	faint
$m/2$ NaCl	+	+	faint
$m/2$ KCl	+	faint	−
$m/3$ $CaCl_2$	−	−	−
$m/3$ $MgCl_2$	−	−	−
$m/2$ (100 NaCl+2.2 KCl)	+	+	faint
$m/2$ (100 NaCl+2 $CaCl_2$)	+	+	faint
$m/2$ (100 NaCl+10 $MgCl_2$)	+	+	faint
$m/2$ (100 NaCl+2.2 KCl+2 $CaCl_2$)	+	+	faint
$m/2$ (100 NaCl+2.2 KCl+10 $MgCl_2$)	+	+	faint
$m/2$ (100 NaCl+2 $CaCl_2$+10 $MgCl_2$)	+	+	faint

The most interesting point brought out in the above table is the independence of these bacteria of a balanced medium. The bacteria live and phosphoresce in pure NaCl without the addition of any bivalent ions. This is true even when the solution is changed three times to remove the last traces of Ca in the bacteria. KCl is also relatively non-toxic, although more so that NaCl. $CaCl_2$ and $MgCl_2$ are very toxic when alone. All combinations of NaCl with the other ions of sea water sustain the bacteria well except that they are neutral media and hence the phosphorescence is dimmed after 24 hours. That pure NaCl should have so little effect on light production is astonishing when we consider its poisonous effect on other marine organisms and tissues, particularly on ciliated cells.

The effect of the alcohols (Table IV.) on light production is very similar to their effect on other life processes: they exert an inhibiting or anæsthetic action which is perfectly reversible. If alcohol solutions containing bacteria which have stopped emitting light are diluted with sea water, light production again begins. As with other tissues the higher the alcohol in the series the greater anæsthetic power it has.

The effect of a number of other substances was studied in a very rough way—namely, by adding a small quantity of the substance to a sea water emulsion of the bacteria in test tubes and then shaking the tubes. With toluol, benzol, ether, chloroform, carbon disulphide, carbon tetrachloride and ethyl butyrate

TABLE IV.

EFFECT OF ALCOHOLS.

Conc. of Alcohol Added to Sea Water.	Light after		
	10 Min.	1 Hr.	24 Hrs.
Methyl alcohol, *2m*	−	−	−
HCH_2OH, 1.5 *m*	+	very faint	−
HCH_2OH, *m*	+	faint	−
HCH_2OH, *m*/2	+	+	+
HCH_2OH, *m*/3	+	+	+
Ethyl alcohol, *m*	−	−	−
CH_3CH_2OH, *m*/1.5	very faint	−	−
CH_3CH_2OH, *m*/2	+	faint	faint
CH_3CH_2OH, *m*/3	+	+	+
Propyl alcohol, *m*/3	−	−	−
$CH_3CH_2CH_2OH$, *m*/4	very faint	−	−
$CH_3CH_2CH_2OH$, *m*/6	faint	very faint	−
$CH_3CH_2CH_2OH$, *m*/8	+	faint	−
$CH_3CH_2CH_2OH$, *m*/15	+	+	+
Isosbutyl alcohol, *m*/10	−	−	−
$(CH_3)_2CHCH_2OH$, *n*/12	very faint	−	−
$(CH_3)_2CHCH_2OH$, *m*/16	+	very faint	−
$(CH_3)_2CHCH_2OH$, *m*/20	+	+	−
$(CH_3)_2CHCH_2OH$, *m*/24	+	+	+
Amyl alcohol, *m*/20	−	−	−
$C_2H_5CH_3CHCH_2OH$, *m*/40	−	very faint[1]	+[1]
$C_2H_5CH_3CHCH_2OH$, *m*/80	very faint	very faint	+[1]
$C_2H_5CH_3CHCH_2OH$, *m*/160	faint	+[1]	+
$C_2H_5CH_3CHCH_2OH$, *m*/320	+	+	+
Capryl alcohol, *m*/400	−	−	−
$CH_3(CH_2)_6CH_2OH$, *m*/800	very faint	very faint	−
$CH_3(CH_2)_6CH_2OH$, *m*/1600	faint	faint	−
$CH_3(CH_2)_6CH_2OH$, *m*/3200	faint	faint	+[1]
$CH_3(CH_2)_6CH_2OH$, *m*/6400	+	+	+
Sea water	+	+	+

[1] Probably due to evaporation of alcohol.

the light was found to disappear almost immediately; with tannin, chloral hydrate, vanillin and sodium glycocholate the light had disappeared in the course of one hour while saponine, amygdalin, and sodium taurocholate had no effect. It is surprising that saponin has no effect on luminous bacteria when we consider its great cytolytic power on other forms in very small concentration.

SUMMARY.

The effects on luminous bacteria of dilution of sea water with water and *m* sugar solution; of HCl and valerianic acid; of NaOH and methyl amine; of the salts of sea water in different combinations; and of methyl, ethyl, propyl, butyl, amyl and capryl alcohol were studied. The points of interest in the results are indicated after each table.